Screening the binding potential of quercetin with parallel, antiparallel and mixed G-quadruplexes of human telomere and cancer protooncogenes using molecular docking approach

Shikhar Tyagi¹ · Sarika Saxena¹ · Priyansh Srivastava¹ · Taniya Sharma¹ · Nikita Kundu¹ · Sarvpreet Kaur¹ · Jadala Shankaraswamy²

Received: 15 January 2020 / Accepted: 17 February 2020 / Published online: 26 February 2020
© Springer Nature Switzerland AG 2020

Abstract

The bioinformatics analysis revealed that more than 400,000 DNA sequences within the human genome have the potential of forming G-quadruplex structures. Specifically, G-quadruplexes have been proved to be involved in the regulation of replication, DNA damage repair, transcription and translation of cancer-related genes and hence a therapeutic target. Targeting G4 with small molecules may regulate its expression. Chemical molecules generally shows more cellular toxicity while natural small molecules are more bioavailable and hence shows high biological activity together with low toxicity. In the present study, we have screened the binding potential of quercetin with parallel, anti-parallel and mixed conformations of telomeric G-quadruplexes, cancer protooncogenes and RNA G-quadruplex using molecular docking approach. Our results suggest that the quercetin mainly binds with grooves of all selected G-quadruplexes and its planar aromatic rings stabilizes the structure by π - π stacking. The binding energies were in a range of -40.24 to -17.11 kcal/mol, -35.73 to -18.09 kcal/mol, -32.68 to -22.47 kcal/mol for telomeric parallel, anti-parallel and mixed G-quadruplexes respectively. Further, binding energies of quercetin with selected cancer proto-oncogenes are in a range of -38.67 to -12.95 kcal/mol and -14.8 and -14.6 kcal/mol for selected RNA G-quadruplex. Hence, this study highlights the comparative differences in binding energies of quercetin even with a group of single conformation of G-quadruplex and helpful to evaluate the binding potential of quercetin to inhibit the activity of telomerases and down-regulate the expression of oncogenes and to be used as a potential anti-cancer agent.

Keywords Docking · Quercetin · G quadruplex · Human telomere · Anti-cancer agent

1 Introduction

G-quadruplexes (G4) are nucleic acid secondary DNA structures that have been found in G-rich regions of genome having biological significance such as human telomeres [1–4] and various oncogenic promoter regions, including c-myc [5–7], c-kit [8, 9], bcl-2 [10, 11] or RET, [12, 13]. The core structures in the G-quadruplex are two or three G-quartets, which can associate through Hoogsteen hydrogen bonding

to form a square planar structure. In addition, the alkali metal ions such as Na^+ and K^+ are known to play important roles in the stabilization of G-quadruplex which coordinate with the eight electronegative O6 atoms of the adjacent stacked G-tetrads. Since K^+ and Na^+ are the physiologically relevant monovalent ions, G-quadruplex formation is favoured under physiological conditions. A G-rich sequence may adopt different structures in the presence of different cations. The K^+ form in general is preferred over Na^+ because of its better

✉ Sarika Saxena, ssaxena1@amity.edu; sarikaigib@yahoo.co.in | ¹Structural Biology Lab, Amity Institute of Biotechnology, Amity University, Sector-125, Expressway Highway, Noida, Uttar Pradesh 201303, India. ²Department of Fruit Science, College of Horticulture, Mojerla, Sri Konda Laxman Telangana State Horticultural University, Hyderabad, Telangana 509382, India.

coordination with eight guanine O6s and a lower dehydration energy as well as its higher intracellular concentration (~ 140 mM) than that of Na⁺ (5–15 mM) [14]. G-rich DNA sequences can form intramolecular G-quadruplexes formed by single-stranded DNA and intermolecular G-quadruplexes between multiple DNA strands [15]. Different sequences can adopt distinct topologies, in addition, a given sequence can fold into a variety of different conformations, as in the case of the human telomeric DNA sequence G3(TAG3)3. In addition, intermolecular multimeric G-quadruplexes can be formed by the association of two or more strands [16]. Intervening mixed-sequence nucleotides form loops of folded G-quadruplex structures which can adopt a variety of different topological forms [17, 18]. The polarity with respect to the two adjacent strands and the location and length of the loops would be expected to lead to a polymorphic G-quadruplex structures. When the alignment of guanine tracts is in same direction, the double-chain reversal (propeller) loops link two adjacent parallel strands to form a parallel structure [1]. When the alignment of guanine tracts is in opposite directions, the edgewise or diagonal loops link two antiparallel strands to form an antiparallel G-quadruplex [19]. When a single strand is oriented in a different direction from the others then it forms antiparallel hybrid or so-called mixed structures [20–22]. Recently, Marušić et al. reported a novel mixed type fold which exhibits a conformation in which all three loop types occur in one conformation: edgewise, diagonal, and double-chain reversal loops [23]. Intramolecular G-quadruplexes formed by single-stranded DNA are of intensive current research interest due to their potential formation in telomeres [24–26] and oncogene promoter sequences [27]. Intramolecular structures form quickly and are more complex, exhibiting great conformational diversity, folding topologies and their loop connectivity. With the extensive and thorough structural studies of G-quadruplexes, numerous rules for folding patterns for G-quadruplex have been recognized. However, to understand each G-quadruplex structure and its ligand interactions, the prediction of a G-quadruplex conformation is necessary although difficult. Reason is high degree of structural polymorphism regulated by various factors like length, base composition of G-rich sequence and physical factors like buffer, pH, and type of binding cation etc. [28–32]. G-quadruplex structures may form intramolecular G-quadruplexes within a single DNA strand, or intermolecular G-quadruplexes between multiple DNA strands [15]. Moreover, due to the various orientations of nucleic acids strands during folding, the G-quadruplex structures can further be divided into different conformations, including parallel, antiparallel, and hybrid conformations [22, 33–35]. Intramolecular G-quadruplexes are of intensive research interest currently

due to their potential of forming G-quadruplex structure in biologically important regions. Telomeric DNA is a 3' G-rich overhang of hexa-nucleotide repeat sequence 5'-(GGGTTA)_n which forms highly polymorphic G-quadruplexes structures depending on the differences in the arrangements of loop, strand orientations, G-tetrad arrangements, and capping structures. Telomere is an attractive anticancer drug targets due to the stabilization of telomere G4s and blockage of telomerase activities and hence offers a new strategy for antitumor therapy. More importantly, telomerase activity is mainly expressed in aging, tumors, cell proliferation, and carcinogenesis that comes around 85–90%. Many researchers have explored many small synthetic drug molecules that induces the formation of G-quadruplex structure of telomere and stabilizes them or may act by binding with oncogenic promoter sequences and down regulate their expression [36, 37]. Synthetic drug molecules are very toxic and have many side effects. On the other hand, nature provides chemically distinct scaffolds in the form of various flavonoids like Quercetin, Myricetin and Genisten etc. which are readily available, less toxic and have better bioavailability [38, 39]. Therefore, from a drug-development point of view, research on screening the binding potential of quadruplex-interactive small natural compounds is expected to be highly active, with a particular emphasis on the selectivity toward polymorphic G-quadruplex structure. In the present study, we attempted to screen the binding potential of plant flavonol (quercetin) with different polymorphic telomeric sequences, cancer proto-oncogene targets and compared with RNA G-quadruplex using molecular docking approach. The purpose of the study is to select the most suitable binding mode of quercetin with respect to the G-quadruplex forming sequences as well as the conformation of G-quadruplex. Based on the calculated binding energies, our results suggest that quercetin binds with high affinity with parallel, antiparallel and mixed telomeric G-quadruplex structures. This highlights that quercetin is well suited natural ligand to target polymorphic telomeric quadruplexes. However, quercetin also binds with cancer protocogenes which indicates that the quercetin can be selected as a natural ligand to down regulate the gene expression of cancer proto-oncogenes. The binding energies for RNA G-quadruplex were in the range of – 14 kcal/mol which indicates that quercetin is not a suitable natural ligand to target the RNA G-quadruplexes in transcriptosome.

2 Materials and methods

2.1 Ligand preparation and virtual screening

The three dimensional structure of Quercetin (PubChem CID: 5280343) was downloaded from the PubChem

Database. Schrödinger LigPrep module was used to generate possible states of quercetin based on variations on ring conformations and tautomers [40]. OPLS 2005 force field was used to generate minimized ionization states at the physiological pH of 7.0 ± 2.0 , as it can influence its binding affinity during interaction studies. Keto-enol tautomerism along with analogous sulphur and nitrogen tautomerization was also done. Eventually, structures were desalted for removal of any water molecules or counter-ions and stereoisomers were generated by retaining the specific chiralities of the original state. A total of 32 structures were generated inclusive of the original state. Initially, all the 32 variants were screened against the pre-processed structure files using GLIDE (i.e. Parallel, Mixed & Anti-Parallel) [41]. The complexes with most negative G scores (ligand binding free energy), were aligned for voting out the best-suited conformation for telomeric G-Quadruplex structures (i.e. Parallel, Anti-Parallel & Mixed) and were isolated in SDF files for MMGBSA (binding energy) estimation.

2.2 Receptor grid generation

All the structure files for Parallel, Mixed and Anti-Parallel were curated from the Protein Data Bank (PDB) and were processed by the removal of excess unwanted waters and cations beyond 5 Angstroms from Hetro groups using Maestro [42]. Missing hydrogens were added and bond orders were assigned to stabilize the raw structure files from PDB. For the NMR structure files, comparative reconstruction using Modeller was done by using the possible states as templates [43]. 3-D structure files of each Quadruplex structure were first optimized at neutral pH and then were minimized using SwissPDB viewer to achieve energetically stable structure for docking [44]. The Gasteiger atomic charges were assigned for all the G4 structures. Receptor grid of $20 \times 20 \times 20$ Angstroms was constructed around the complexes in three dimensions to be sufficiently large to encompass the entire G4 structures using the Receptor Grid Generation module [40].

2.3 Molecular docking and MMGBSA calculations

Schrödinger Glide with OPLS 2003 force field was used with Xtra precision workflow to carry out the Molecular docking [45]. The scaling factor was set to the default of 0.80 with the partial charge cutoff at 0.15 and root mean square deviations for ligand geometries were also computed. We flexibly docked categorically isolated (i.e. Parallel, Anti-Parallel & Mixed) conformations of the quercetin with their respective categorical structure files (i.e. Parallel, Anti-Parallel & Mixed). Docking score for every receptor-ligand was calculated for setting up the Molecular Mechanics generalized Born and surface area continuum

solvation (MM/PBSA) estimation. MMGBSA was calculated in VSGB to estimate the free energy of the binding of quercetin to telomeric G-Quadruplexes [40, 41, 45–47]. After, sorting the ligand-receptor poses based on Gibbs Free Energy (ΔG), bound states of quercetin were again superimposed to find the similarity of binding conformation between the member of Parallel, Anti-Parallel & Mixed telomeric G-Quadruplexes structures independently.

All figures were rendered with PyMOL (www.pymol.org).

2.4 Circular dichroism spectroscopy

CD spectra were carried out on JASCO-715 spectropolarimeter using a quartz cuvette of 1 cm path length. All the spectra were recorded in the range of 200–350 nm wavelengths at a scanning rate of 100 nm min^{-1} . Before measurement, the samples were heated to 95°C in water bath and slowly cooled till water attains room temperature and incubated at 4°C overnight to avoid any non-equilibrium structures. Average scans of the DNA samples were subtracted from the buffer scan and data was normalized as a function of DNA strand concentration and pathlength of the cuvette. The CD curve was plotted between ellipticity as a function of wavelength.

2.5 Thermal melting analysis

UV absorbance of different samples were recorded with a Shimadzu 1800 spectrophotometer (Shimadzu, Tokyo, Japan) equipped with a temperature controller. Melting curves of DNA structures were obtained by measuring the UV absorbance at 295 nm in buffer pH 7.0 [100 mM KCl, and 0.5 mM EDTA] in the presence or absence of quercetin at DNA:quercetin ratio (1:0), (1:1), (1:2), (1:5), and (1:10). The T_m values for $4 \mu\text{M}$ DNA structures were obtained from the UV melting curves as described previously¹⁷. The heating rates were $0.5^\circ\text{C min}^{-1}$. The thermodynamic parameters were evaluated from the fit of the melting curves to a theoretical equation for an intramolecular association as described previously [48, 49]. Before measurement, the samples were heated to 95°C in water bath and slowly cooled till water attains room temperature and incubated at 4°C overnight to avoid any non-equilibrium structures. Experiment has been repeated in triplicates to reproduce the data.

3 Results and discussion

Guanine-rich nucleic acid sequences can fold into diverse topologies depending upon the length of the G tract, loop length and the syn- and anti-conformation of Guanines.

Notably, not only the sequences but also the surrounding conditions determine the conformation of DNA G-quadruplexes. In particular, different monovalent and divalent cations coexisting in solution are known to induce diverse G-quadruplex structures with distinct thermodynamics [1, 19, 20, 50]. Hence, first we retrieved the crystal structures of different telomeric sequences and their PDBs from NCBI database and segregated them based on the folding topology into parallel, antiparallel and mixed G-quadruplexes. To understand better the binding mode of quercetin in detail we have also retrieved the G-quadruplex sequences of available human proto-oncogenes as well as one sequence of RNA G-quadruplex. All selected DNA and RNA sequences along with their PDB ID's are given in Table 1.

3.1 Superimpose conformers of Quercetin

As we have chosen to compare the binding efficiencies of quercetin with different topologies of G-quadruplex. Therefore, to make it more systematic, first we tried to identify the superimpose conformers of quercetin for parallel G-quadruplex, anti-parallel G-quadruplex as well as

for mixed G-quadruplex separately as shown in Fig. 1 using Maestro alignment tool of Schrodinger software. The basic idea was to identify structure of most relevant conformers of the quercetin molecule based on the smart patterns for the atoms with each distinct G-quadruplex topology and to understand the conformational change using the software based drug discovery approach.

3.2 Comparative analysis of molecular docking studies of parallel g-quadruplex structures with quercetin

Molecular docking calculations was performed to investigate the binding mode of quercetin with Human Parallel telomeric G4 (1KF1) using Schrodinger. The details of atoms involved in hydrogen bonding between the parallel G-quadruplexes with Quercetin are summarized in Table 2. The crystal structure of 1KF1 was used as a template processed by removal of excess unwanted waters and cations beyond 5 Angstroms from Hetro groups using Maestro [42]. Here, during the initial optimization, the planarity restriction was not applied, and the final structures are obtained at intrinsic planar and nonplanar arrangements.

Table 1 Retrieval of G-quadruplex forming sequences of telomeres, cancer proto-oncogenes and RNA G-quadruplex from NCBI data base (<https://www.ncbi.nlm.nih.gov/Nucleotide>)

S. no	Name	DNA/RNA sequence	Cation	PDB accession ID	Conformation	Score	dG (MMGBSA)
1	Human Telomeric DNA	d[AGGG(TTAGGG)3]	K ⁺	1KF1	Parallel	−6.78	−40.24
2	Human Telomeric DNA	d(TAGGGTTAGGGT)	K ⁺	1K8P	Parallel	−5.59	−33.06
3	Human Telomeric DNA	d(TG4T)	Na ⁺	352D	Parallel	−6.46	−31.93
4	Human Telomeric DNA	d(TAGGGTTAGGGT)	Na ⁺ , K ⁺	3CCO	Parallel	−6.16	−29.54
5	Human Telomeric DNA	d(TAGGGTTAGGGTTAGGGTTAGGG)	K ⁺	3CDM	Parallel	−6.53	−23.17
6	Human Telomeric DNA	d(TGGGGT)	Na ⁺	1O0K	Parallel	−4.75	−17.11
7	Human Telomeric DNA	d[(GGGTTA)3GGGT]	K ⁺	2KF8	Antiparallel	−6.05	−35.73
8	Human Telomeric DNA	d(gcGA[G]1Agc)	K ⁺	1V3N	Antiparallel	−6.20	−29.27
9	Human Telomeric DNA	d(gcGA[G]1Agc)	K ⁺	1V3O	Antiparallel	−6.39	−21.12
10	Human Telomeric DNA	d(gcGA[G]1Agc)	K ⁺	1V3P	Antiparallel	−6.00	−18.09
13	Human Telomeric DNA	d[TAG3(T2AG3)3TT]	K ⁺	2JSL	(3 + 1) mixed	−6.15	−32.68
14	Human Telomeric DNA	d[A3G3(T2AG3)3AA]	K ⁺	2HY9	(3 + 1) mixed	−7.12	−30.85
15	Human Telomeric DNA	d(GGGTTAGGGTTAGGGT)	Na ⁺	2AQY	(3 + 1) mixed	−5.39	−28.67
16	Human Telomeric DNA	d[TTGGG(TTAGGG)3A]	K ⁺	2GKU	(3 + 1) mixed	−5.7	−24.79
17	Human Telomeric DNA	d[(T2AG3)4TT]	K ⁺	2JPZ	(3 + 1) mixed	−4.42	−23.13
18	Human Telomeric DNA	d[TAGGG(TTAGGG)3]	K ⁺	2JSM	(3 + 1) mixed	−4.97	−22.47
19	c-myc	d(TGAGGGTGGGTAGGGTGGGTAN)	K ⁺	1XAV	Parallel	−8.07	−38.67
20	c-kit2	d(CGCGCGGGCGGAGGGAGGGT)	K ⁺	2KQH	Parallel	−7.39	−24.75
21	Human c-Myc Promoter	TGAGGGTGGGTAGGGTGGGTAA	K ⁺	6AU4	Parallel	−8.36	−25.38
22	human VEGF promoter	d(CGCGGGCGGGCTTGGGCGGGT)	K ⁺	2M27	Parallel	−5.61	−12.95
23	RNA tetraplex	(TGTGGT)	K ⁺	1P79	Parallel	−6.13	−14.8
24	RGG-motif of FMRP	d(GCTGCGGTGTGAAGGAGTGGCTGGGTTGCGCAGC)	K ⁺	5DE5	(3 + 1) mixed	−9.06	−14.6

Fig. 1 All quercetin conformers in Parallel G-quadruplex sequences (a) Antiparallel G-quadruplex sequences (b) and Mixed G-quadruplex sequences (c)

The size of grid box was $20 \times 20 \times 20$ in three dimensions. The grid was set to be sufficiently large to cover significant portions of the active sites and the result is depicted in Fig. 2a. After the optimization of the quercetin conformer for the parallel G-quadruplex, it interacted with G4. Results suggest that each G-tetrad is stabilized by Hoogsteen hydrogen bonding between hydrogen bond donor and acceptor atoms. Quercetin adopts a groove binding conformation in which hydrogen atom of the C-2 atom with A7, C-8 forms a hydrogen bond with T5. Another two hydrogen bonds formed between oxygen atom of C-13 and hydrogen atom of C-14 of quercetin with G4 and with G10 respectively (Table 1). In drug designing, the calculation of binding energies is an important parameter to evaluate the affinity of binding of the drug with G4. We calculated free energy of the binding of quercetin to telomeric G-Quadruplexes using the Molecular Mechanics generalized Born and Surface area which was found to be -40.24 kcal/mol (Table 1). The negative sign of ΔG indicates the favourable binding of quercetin with 1KF1 which is due to the formation of three hydrogen bonds formation during the interaction of quercetin with 1KF1. Next, we compared the binding mode of quercetin with 1K8P which is another parallel telomeric G-quadruplex formed in the presence of K^+ [51] (Fig. 2b). As it can be seen in figure that quercetin binds in groove in which hydrogen atom of the C-7 atom of quercetin forms hydrogen bond with G3 and oxygen atom of C-8 atom. Second hydrogen bond is established between the hydrogen atoms of C-13 with G5. The estimated binding energies for the quercetin binding with 1K8P was estimated as -33.06 kcal/mol (Table 1). Next, we checked the binding mode of another parallel telomeric G-quadruplex 352D (Fig. 2c). Here, quercetin during the interaction forms five hydrogen

bonds with G4. Three hydrogen bond formed between hydrogen atom of C-2, C-7 and C-8 of quercetin with G4 (annotated by software as G25 in Fig. 2). Another two hydrogen bonds also formed between hydrogen atom of C-6 and oxygen atom of C-8 of G5 (annotated by software as G35 in Fig. 2). The binding energies calculated for this interaction studies -31.93 kcal/mol (Table 1). Next, we compared another parallel G-quadruplex (3CCO) for its binding mode with quercetin (Fig. 2d). As can be seen in the figure that quercetin forms six hydrogen bonds. During 3CCO and quercetin interaction, hydrogen bond formed between the hydrogen atom of C2, C6, C8, C13, C14 and oxygen atom of C13 of quercetin bonded with A8 (annotated as A1008 in Fig. 1d), with G9 (annotated as A1009 in Fig. 2d), with G11 (annotated as A1011 in Fig. 2d) and oxygen atom of C13 of quercetin bonded with G5 (annotated as G1005 in Fig. 2d). The binding energies of this interaction calculated as -29.54 kcal/mol (Table 1). We checked the binding of another parallel structure (3CDM) to find out the binding site of quercetin (Fig. 2e). As can be seen in the figure that 3CDM formed five hydrogen bonds between the hydrogen atom of C-2, C-6, C-7, C-13 and C-14 with G15, G3, G15, G9 and G3. The binding energies of this interaction studies calculated as -23.17 kcal/mol (Table 1). Further, another parallel G4 (100K) was docked with quercetin (Fig. 2f). During the interaction this G4 formed three hydrogen bonds by involving hydrogen atom of C1, C13 and C14 of quercetin with G11 and G10 (annotated as G1011 and G1010 in Fig. 2f) respectively. The binding energies for this interaction is calculated as -17.11 kcal/mol (Table 1). This data highlights the important points like quercetin binds with parallel telomeric G-quadruplex mainly by groove binding. Secondly, the quercetin recognized and binds with parallel G-quadruplex in a distinct manner.

Table 2 Retrieval of G-quadruplex forming sequences of telomeres, cancer proto-oncogenes and RNA G-quadruplex from NCBI data base (<https://www.ncbi.nlm.nih.gov/Nucleotide>)

S. no.		Identifier	Quercetin	Nucleotide	Nucleotide sequence
1	Parallel	1KF1	H of C2	A7	d[AGGG(TTAGGG)3]
			H of C8	T5	
			O of C13	G4	
			H of C13	G10	
			H of C14	G10	
2		1K8P	H of C7	G3	d[TAGGGTTAGGGT]
			O of C8	G3	
			H of C13	G5	
3		352D	H of C2	G4 (annotated as G25)	d[TG4T]
			O	G5 (annotated as G35)	
			H of C6	G5 (annotated as G35)	
			H of C7	G4 (annotated as G25)	
			H of C8	G4 (annotated as G25)	
4		3CCO	H of C2	A8 (annotated as A1008)	d[TAGGGTTAGGGT]
			H of C6	G9 (annotated as G1009)	
			H of C8	G10 (annotated as G1010)	
			H of C13	G11 (annotated as G1011)	
			O of C13	G5 (annotated as G1005)	
5		3CDM	H of C14	G11 (annotated as G1011)	d[TAGGGTTAGGGTTAGGGTTAGGG]
			H of C2	G15	
			H of C6	G3	
			H of C7	G15	
			H of C13	G9	
6		100K	H of C14	G3	d[TGGGGT]4
			H of C1	G11 (annotated as G1011)	
			H of C13	G10 (annotated as G1010)	
7		2KF8	H of C14	G10 (annotated as G1010)	d[(GGGTTA)3GGGT]
			H of C6	G21	
			H of C7	G14	
			H of C8	G14	
			H of C13	G20	
8		1V3N	H of C14	G20	d[GCGA(G)1AGC]
			H of C2	G5	
			H of C6	A6	
			H of C7	G5 (annotated as G13)	
			H of C8	G5 (annotated as G13)	
9	Antiparallel	2JSL	H of C13	G7	d[TAG3(T2AG3)3TT]
			H of C14	G7	
			H of C2	G3	
			H of C7	G3	
			H of C13	A2	
10		2HY9	H of C14	T1	d[A3G3(T2AG3)3AA]
			H of C2	G11	
			H of C6	G10	
			O of C13	A3	

Table 2 (continued)

S. no.		Identifier	Quercetin	Nucleotide	Nucleotide sequence
11		2AQY	H of C1 H of C3 H of C7 H of C8 H of C13 H of C14	G7 T16 G7 G7 A6 A6	d[GGGTTAGGGTTAGGGT]
12		2GKU	H of C2 H of C6 H of C13 H of C14	T7 T6 G11 G11	d[TTGGG(TTAGGG)3A]
13		2JPZ	H of C6 H of C8	A3 G22	d[(T2AG3)4TT]
14		2JSM	H of C2 H of C7 H of C8 H of C13 H of C14	A2 G3 G3 A2 T6	d[TAGGG(TTAGGG)3]
15	Mixed	1XAV	O of C6 H of C6 O H of C8 H of C13	G9 G13 G10 G15 A22	d[TGAGGGTGGGTAGGGTGGGTAN]
16		2KQH	H of C2 H of C6 O of C7 H of C8 H of C13 H of C14	G14 G12 G7 G8 G15 G15	d[CGGGCGGGCGCGAGGGAGGGT]
17		6AU4	H of C2 H of C6 O H of C14	A21 A22 A21 A21	d[TGAGGGTGGGTAGGGTGGGTAA]
18		2M27	H of C2 H of C7 H of C8 H of C14 O of C14	G15 G15 C10 C10 G9	d[CGGGGCGGGCCTTGGGCGGGT]
19		5DE5	H of C2 H of C6 H of C8 O of C8 H of C13 H of C14	G4 G31 G4 C5 C5 C5	d[GCTGCGGTGTGGAAGGAGTGG CTGGGTTGCGCAGC]
20		4H29	H of C2 H of C6	G17 G13	d[GGGCGGGGAGGGGAAGGGA]
21	RNA	IP79	H of C6 H of C7 O of C7 H of C8	G4 G5 U6 U6	d[TGTGGT]

We observed the differences in the binding modes of quercetin with parallel conformation of G-quadruplexes, confirmed by the difference in the binding energies of the various complexes discussed above. We propose that the differences in binding energies is mainly due to the differences in the bond length of the hydrogen bond, shorter the bond length more will be the stability and longer the bond length less will be the stability.

3.3 Comparative analysis of molecular docking studies of anti-parallel g-quadruplex structures with quercetin

We have selected two telomeric crystal structures which forms antiparallel G-quadruplexes 2KF8 [52] and 1V3N [53] and docked with quercetin. The details of atoms involved in hydrogen bonding between the anti-parallel G-quadruplexes with Quercetin are summarized in Table 2.

Figure 3a shows the binding mode of 2KF8 with quercetin in which hydrogen atom of C-6, C-7, C-8, C-13 and C-14 formed bond with G21, G14, G14, G20 and G20 respectively. The binding energies for this interaction in calculated as -35.73 kcal/mol (Table 1). Next, we compared another crystal structure 1V3N docked with quercetin (Fig. 3b) in which hydrogen of C-2, C-6, C-7, C-8, C-13 and C-14 formed bond with G5, A6, G5 (annotated as G13 in Fig. 3b), G5 (annotated as G13 in Fig. 3b), G7 and G7 respectively. Here, the binding energies calculated for this interaction studies were -29.27 kcal/mol (Table 1). This comparative study highlights the distinctive recognition of quercetin due to its bended structure and the glycosidic conformation of the guanine base and the differences in the bond length.

3.4 Comparative analysis of molecular docking studies of mixed G-quadruplex structures with Quercetin

Next, we analysed the different telomeric crystal structures of mixed G-quadruplex. The details of atoms involved in hydrogen bonding between the mixed G-quadruplexes with Quercetin are summarized in Table 2. First crystal structure selected for mixed telomeric G-quadruplex was 2JSL which was docked with quercetin (Fig. 4a). Here, the hydrogen atom of C2, C7, C13 and C14 bonds with G3, G3, A2 and T1 respectively. The calculated binding energies of this interaction was -32.68 kcal/mol (Table 1). Further, another mixed G-quadruplex (2HY9) was docked with quercetin (Fig. 4b) to know the details of binding sites and their binding energies. We observed that hydrogen atom of C2, C6 and oxygen atom of C13 forms bond with G11, G10 and A3 respectively. The binding energies

calculated for this interaction— 30.85 kcal/mol (Table 1). Next, another mixed G-quadruplex (2AQY) was docked with quercetin and it was observed that quercetine binds with groove (Fig. 4c). During binding the hydrogen atom of C-1, C-3, C-7, C-8, C-13 and C-14 of quercetin bonded with G7, T16, G7, G7, A6 and A6 of 2AQY mixed G-quadruplex respectively. The binding energies of this interaction was calculated as -28.67 kcal/mol (Table 1). Next, we performed the molecular docking with another mixed G-quadruplex (2GKU) with quercetin (Fig. 4d). It was observed that hydrogen atom of C-2, C-6, C-13 and C-14 of quercetin formed hydrogen bonds with T7, T6, G11 and G11 of 2GKU respectively. The binding energies for this interaction was calculated as -24.79 kcal/mol (Table 1). Further, another mixed G-quadruplex (2JPZ) was docked with quercetin (Fig. 4e). It was found that quercetin binds with grooves of G4 with available binding sites in which hydrogen atom of C-6 and C-8 of quercetin bonded with A3 and G22 respectively. The binding energies calculated as -23.13 kcal/mol. Next, another G-quadruplex (2JSM) was docked with quercetin (Fig. 4f). It was found that during binding in the major groove of 2JSM, hydrogen atom of C-2, C-7, C-8, C-13, C-14, and oxygen and hydrogen atoms of C-6 of quercetin bound with A2, G3, G3, A2, T9, G9 and G13 respectively. The binding energies of this interaction energies was calculated as -22.47 kcal/mol (Table 1).

3.5 Comparative analysis of molecular docking studies of cancer proto-oncogenes with quercetin

We also screened the binding potential of quercetin with G-quadruplex structure formed by c-myc proto-oncogene (1XAV) which forms parallel G-quadruplex (Fig. 5a). The details of atoms involved in hydrogen bonding between the G-quadruplexes formed by cancer proto-oncogenes with Quercetin are summarized in Table 2. Our molecular docking results suggested that oxygen and hydrogen atom of C-6, oxygen atom of C-1 and hydrogen atom of C-8 and C-13 formed hydrogen bond with G9, G13, G10, G15 and A22 respectively (Fig. 5a). The binding energies calculated for this interaction energies were -38.67 kcal/mol (Table 1). Next, we screened c-kit2 which forms parallel G-quadruplex in K^+ (2KQH) which binds with quercetin with its grooves in which hydrogen atom of C-2, C-6, C-8, C-13, C-14 and oxygen atom of C-7 with G14, G12, G8, G15, G15 and G7 respectively (Fig. 5b). The binding energies calculated for this interaction energies were -24.75 kcal/mol (Table 1). Further, we screened the binding potential of

Fig. 2 Lowest—energy-docked conformations representing the interaction profiles of quercetin with Parallel G-quadruplexes. The binding parameters are given in Table 1

Fig. 3 Lowest—energy-docked conformations representing the interaction profiles of quercetin with Anti-Parallel G-quadruplexes. The binding parameters are given in Table 1

quercetin with Human c-myc promoter which formed parallel G-quadruplex in K^+ (6AU4) (Fig. 5c). Here, hydrogen atom of C-2, C-6, hydrogen atom of C-14 and oxygen atom of C-1 formed hydrogen bond with A21, A22 and A21 respectively. The binding energies calculated for this interaction energies were -25.38 kcal/mol (Table 1). Next, we docked another Parallel G-quadruplex formed by VEGF promoter (2M27) with quercetin (Fig. 5d). We observed that quercetin binds with grooves of 2M27 in which hydrogen atom of C-14, C-2, C-7, C-8, and hydrogen and oxygen atom of C-14 with A21, G15, G15, C10, C10 and G9 respectively. The binding energies calculated for this interaction were -12.97 kcal/mol (Table 1). These results are consistent with previously published reports of quercetin binding with c-myc promoter which stated that quercetin stacks at a 5' and 3'-G-tetrads of c-myc

promoter G-quadruplex, stabilized the structure by π - π interactions [54, 55].

3.6 Comparative analysis of molecular docking studies of RNA G-quadruplex with quercetin

To confirm whether quercetin may bind to RNA G-quadruplex or not, we selected two RNA G-quadruplex forming motifs one is 1P79 and another one is RGG-motif of FMRP (PDB I.D. 5DE5) docked with quercetin to know the binding sites and binding energies. The details of atoms involved in hydrogen bonding between the RNA G-quadruplexes with Quercetin are summarized in Table 2. Figure 6a showed the molecular docking results of 1P79 with quercetin in which hydrogen atom of C-6, C-7, C-8 and oxygen atom of C-7 bonded with G4, G5, U6

Fig. 4 Lowest—energy-docked conformations representing the interaction profiles of quercetin with Mixed G-quadruplexes. The binding parameters are given in Table 1

and U6 respectively. The binding energies calculated for this interaction were -14.8 kcal/mol (Table 1). Next, we docked another RNA quadruplex (5DE5) with quercetin (Fig. 6b). Binding results suggested that hydrogen atom of C-2, C-6, C-8, C-13, C-14 and oxygen atom of C-8 forms hydrogen bond with G4, G31, G4, C5, C5, and C5. The binding energies calculated for this interaction were -14.6 kcal/mol (Table 1). The obtained results shows that the quercetin drug interacted with RNA G-quadruplex but interaction energies are less and close to similar energy values for both the RNA G-quadruplexes.

3.7 Structural studies on the binding of quercetin with Human Telomeric and Bcl-2 G-quadruplex structures with and without quercetin in the presence of 100 mM K^+ under molecular crowding conditions

CD spectroscopy was employed to investigate the changes on the conformation of 23-mer IND DNA sequence 5'-d(TAGGGTTAGGGTTAGGGTTAGGG)-3' upon quercetin binding. The structure of each DNA strand is in 30 mM sodium cacodylate buffer pH (7.0), 100 mM K^+ and 40 wt%

PEG 200 in presence and absence of different concentrations of quercetin (Fig. 7a). CD spectrum of IND is characterized by a positive peak at 292 nm and negative peak at 240 nm typically observed for an antiparallel G-quadruplex in the presence of K^+ [56]. Next, IND was titrated with an increasing concentration 0.2 μ M, 0.4 μ M, 0.8 μ M, 2 μ M, 4 μ M and 8 μ M of quercetin. We observed a slight increment of CD intensity at 292 nm and small shoulder around 260 nm and the negative peak at 240 nm shifted towards 236 nm upon the titration of quercetin. However, these changes are very small but the overall CD spectra showed that quercetin binds with IND G-quadruplex. In contrast, HTPu in the presence of K^+ (Fig. 7b), exhibits a strong positive peak around 290 nm with a minor shoulder around 260 nm, 219 nm and a small negative peak at 234 nm, indicating a formation mixed G-quadruplex, consistent with the previously published report [56]. On titrating HTPu with quercetin, we observed slight increment of CD signal at 290 nm and shoulder at 260 nm became sharper with an increase in intensity at 219 nm. These changes indicate that the binding of quercetin inducing structural change. Next, we recorded the CD spectra of Bcl2 in similar solution conditions and titrated with an increasing concentration

Fig. 5 Lowest—energy-docked conformations representing the interaction profiles of quercetin with G-quadruplexes formed by cancer proto-oncogenes. The binding parameters are given in Table 1

of quercetin. We observed prominent negative peak at 298 nm, 219 nm and negative peak at 233 nm indicating a formation of antiparallel G-quadruplex [50, 57] (Fig. 7c). After the addition of quercetin, we observed a decrease in CD intensity at 298 nm and shoulder towards 260 nm started emerging. There was also increase in CD intensity at 219 nm while negative peak in control without quercetin at 233 nm shifted towards 237 nm. We proposed that the gradual decrease in CD intensity on increasing the quercetin concentration at 298 nm and development of the emerging shoulder at 260 nm shows the structural transition from antiparallel to parallel G-quadruplex, although this may require extended studies with more increasing concentration of quercetin to reach on any conclusion. These observed changes are due to the quercetin binding may be due to the two way binding of the quercetin with G-quadruplex structure, one is hydrogen bonding involving the hydrogen bonding sites of quercetin with G-bases and another is due to the intercalation of the

aromatic ring within G-quartet core, consistent with the previously published reports [59].

3.8 Thermodynamic analysis of the Human Telomeric and Bcl2 G-quadruplex with and without Quercetin

Next, we explored the thermal stability of the DNA structures with and without quercetin. Figure 8a shows normalized UV melting profile of 4 μ M IND in the buffer containing 100 mM KCl and 40 wt% PEG 200 in the absence and presence of quercetin. The ratios of DNA:quercetin were (1:0, 1:0.1, 1:0.2, 1:1, 1:2 and 1:3) respectively (Fig. 3). The melting temperature (T_m) was evaluated by a curve fitting procedure as described previously [48, 49]. The T_m of the IND G4 was increased from 67.3 $^{\circ}$ C, 69.1 $^{\circ}$ C, 70.3.0 $^{\circ}$ C, 70.3 $^{\circ}$ C, 70.3, 71.5 and 75.6 $^{\circ}$ C respectively. The melting curves with a single transition and overall 8.3 $^{\circ}$ C difference in the T_m values in different DNA:quercetin ratios.

Fig. 6 Lowest—energy-docked conformations representing the interaction profiles of quercetin with G-quadruplexes formed by RNA G-quadruplex. The binding parameters are given in Table 1

These results are consistent with the previously published reports on the stabilization of telomeric G-quadruplex on the quercetin binding [58, 59]. Next, we recorded the data with HTPu G4 in the buffer containing 100 mM KCl and 40 wt% PEG 200 in the absence and presence of quercetin with similar ratios. T_m of the HTPu G4 was varied from (68.5 °C, 69.0 °C, 69.0 °C, 69.0 °C, 69.0 and 69.5 °C) respectively (Fig. 8b). These results indicated that overall change in 1 °C these G4s possess similar thermal stability upon quercetin binding. On the other hand, the T_m value of the Bcl-2 G4 was increased from 65.9 °C, 66.5 °C, 67.5 °C, 68.0 °C, 68.0 °C and to 68.5 °C in similar solution conditions respectively (Fig. 8c). Therefore, the overall stabilization in T_m was of 3 °C upon quercetin binding, although further systematic studies are required. These results are consistent with our CD results which state the binding of antiparallel G4 with quercetin and previously published reports [60–64].

To assess the origin of the observed stabilities of IND, HTPu and Bcl-2 G4 upon the complex formation with quercetin, the thermodynamic parameters of their formations, such as the enthalpy change (ΔH°), the entropy change (ΔS°), and the free energy change at 25 °C (ΔG°_{25}) of the IND, HTPu and Bcl-2 G4 formation were estimated in the presence and absence of (1:0, 1:0.1, 1:0.2, 1:1, 1:2 and 1:3) (summarized in Table 3). The interaction of IND-quercetin complex on increasing the quercetin concentration in a buffer containing 100 mM KCl and 30 mM sodium cacodylate buffer (pH 7.0), ΔH° decreased -37.17 kcal/mol, -47.50 kcal/mol, -48.15 kcal/mol, -50.75 kcal/mol, -54.88 kcal/mol, -57.04 kcal/mol, $T\Delta S^\circ$ decreased from -12.76 kcal/mol, -16.18 kcal/mol, -16.40 kcal/mol, -17.20 kcal/mol, -18.69, -19.39 kcal/mol.

The free energy (ΔG°) at 298 K follows the same order. ΔG°_{25} decreased -0.27 kcal/mol, -0.28 kcal/mol, -0.30 kcal/mol, -0.35 kcal/mol, -0.38 kcal/mol, -0.51 kcal/mol (Table 3). The interaction of HTPu-quercetin complex on increasing the quercetin concentration in a buffer containing 100 mM KCl and 30 mM sodium cacodylate buffer (pH 7.0), ΔH° decreased -48.81 kcal/mol, -49.84 kcal/mol, -51.50 kcal/mol, -56.52 kcal/mol, -56.67 kcal/mol, -73.19 kcal/mol, $T\Delta S^\circ$ decreased from -16.67 kcal/mol, -17.00 kcal/mol, -17.58 kcal/mol, -19.34 kcal/mol, -19.39, -25.00 kcal/mol. The free energy (ΔG°) at 298 K follows the same order. ΔG°_{25} decreased -0.28 kcal/mol, -0.26 kcal/mol, -0.26 kcal/mol, -0.33 kcal/mol, -0.36 kcal/mol, -0.41 kcal/mol respectively (Table 3). However, the interaction of Bcl2-quercetin complex on increasing the quercetin concentration in a buffer containing 100 mM KCl and 30 mM sodium cacodylate buffer (pH 7.0). ΔH° decreased -29.17 kcal/mol, -31.31 kcal/mol, -33.40 kcal/mol, -38.33 kcal/mol, -45.21 kcal/mol, -45.88 kcal/mol, -51.99 kcal/mol, $T\Delta S^\circ$ decreased from -10.17 kcal/mol, -10.64 kcal/mol, -11.40 kcal/mol, -13.11 kcal/mol, -15.72 kcal/mol, -15.73 kcal/mol and -17.83 kcal/mol (Table 3). Therefore, These thermodynamic parameters suggested that the stabilization of the G-quadruplex by the binding of quercetin is promoted by a favourable an enthalpic contribution exceeding an unfavorable entropy change. Accordingly, specific intermolecular hydrogen bonding between the quercetin and G4, as well as the stacking interactions of aromatic rings may contribute this enthalpic stabilization of G4. These enthalpic stabilization effects on G4 derived from specific interactions have been reported for small molecular ligands effects G4s [50, 56–64].

Fig. 7 CD spectra of normalized molar ellipticity of 4 μ M IND (**a**), 4 μ M HTPu (**b**) and 4 μ M Bcl-2 (**c**) in buffer containing KCl (100 mM), 0.5 mM EDTA without any additive (black line) and titrated with

an increase in concentration of quercetine. Control DNA of each sequence along with highest concentration of quercetin used in this study is shown in inset separately

Fig. 8 UV melting curves of 4 μM IND (**a**), 4 μM HTPu (**b**) and 4 μM Bcl-2 (**c**) in buffer containing KCl (100 mM), 0.5 mM EDTA without any additive (green line) and titrated with an increase in concentration of quercetin. in buffer containing KCl (100 mM), 0.5 mM EDTA, without any additive (light green line); with 0.2 μM querce-

tin (black), 0.4 μM quercetin (red), 0.8 μM quercetin (pink), 2 μM quercetin (green), 4 μM quercetin (magenta), 8 μM quercetin (blue) respectively. Experiment has been repeated in triplicates to reproduce the data

Table 3 Thermodynamic parameters for IND interaction with quercetin in the presence of 100 mM K^+ and 40 wt% PEG 200

Abbreviations	$T_m/^\circ\text{C}$	$\Delta G^\circ 25/\text{kcal mol}^{-1}$	$\Delta H^\circ/\text{kcal mol}^{-1}$	$T\Delta S^\circ/\text{kcal mol}^{-1}$
IND:Quercetin (1:0)	65.9	-0.27 ± 0.2	-37.71 ± 0.6	-12.76 ± 1.2
IND:Quercetin (1:0.1)	66.5	-0.28 ± 0.1	-47.50 ± 0.2	-16.18 ± 2.0
IND:Quercetin (1:0.2)	67.5	-0.30 ± 0.2	-48.15 ± 0.3	-16.40 ± 1.6
IND:Quercetin (1:1)	68.0	-0.35 ± 0.3	-50.75 ± 2.2	-17.20 ± 1.8
IND:Quercetin (1:2)	68.0	-0.38 ± 0.2	-54.88 ± 1.2	-18.69 ± 1.4
IND:Quercetin (1:3)	68.5	-0.51 ± 0.2	-57.08 ± 1.4	-19.39 ± 1.6
Thermodynamic parameters for HTPu interaction with quercetin in the presence of 100 mM K^+ and 40 wt% PEG 200				
HTPu:Quercetin (1:0)	68.5	-0.28 ± 0.2	-48.81 ± 0.6	-12.76 ± 1.2
HTPu:Quercetin (1:0.1)	69.0	-0.26 ± 0.1	-49.84 ± 0.2	-16.18 ± 2.0
HTPu:Quercetin (1:0.2)	69.0	-0.26 ± 0.2	-51.50 ± 0.3	-16.40 ± 1.6
HTPu:Quercetin (1:1)	69.0	-0.32 ± 0.3	-56.52 ± 2.2	-17.20 ± 1.8
HTPu:Quercetin (1:2)	69.0	-0.36 ± 0.2	-56.67 ± 1.2	-18.69 ± 1.4
HTPu:Quercetin (1:3)	69.5	-0.42 ± 0.2	-73.10 ± 1.4	-19.39 ± 1.6
Thermodynamic parameters for Bcl2 interaction with quercetin in the presence of 100 mM K^+ and 40 wt% PEG 200				
Bcl2:Quercetin (1:0)	67.3	-0.14 ± 0.2	-29.70 ± 0.6	-10.17 ± 1.2
Bcl2:Quercetin (1:0.1)	69.1	-0.18 ± 0.1	-31.31 ± 0.2	-10.64 ± 2.0
Bcl2:Quercetin (1:0.2)	70.3	-0.22 ± 0.2	-33.40 ± 0.3	-11.40 ± 1.6
Bcl2:Quercetin (1:1)	70.3	-0.25 ± 0.3	-38.33 ± 2.2	-13.11 ± 1.8
Bcl2:Quercetin (1:2)	71.5	-0.29 ± 0.2	-45.88 ± 1.2	-15.73 ± 1.4
Bcl2:Quercetin (1:3)	75.6	-0.31 ± 0.2	-51.99 ± 1.4	-17.83 ± 1.6

All experiments between two telomeric DNA sequences (named as IND and HTPu) and another cancer protooncogene (bcl-2) with quercetin were carried out in a buffer containing 30 mM sodium cacodylate buffer (pH 7.0), 100 mM KCl, 40 wt% PEG 200 and 0.5 mM EDTA. Thermodynamic parameters are the average values obtained from melting curves replicated three times

4 Conclusion

In this study, we have examined the binding sites of flavonoid (quercetin) with polymorphic telomeric G-quadruplex DNA structures (parallel, antiparallel and mixed) and

calculated their binding energies. We also examined the binding potential of quercetin with cancer proto-oncogenes and with RNA G-quadruplexes. The binding energies of quercetin calculated for each structure separately for each G-quadruplex structure gives an idea that Quercetin

may be used as a lead molecule to target polymorphic telomeric G-quadruplex as well as cancer proto-oncogenes and hence a suitable natural drug molecule for anti-cancer therapeutics. Our study highlighted the structural aspects of the binding of Quercetin with different polymorphic biological targets of G-quadruplexes and we believe that this is the first report for the identification of selection of suitable nucleic acid target for quercetin binding.

Funding This work was supported by the research grant of “Department of Biotechnology (DBT)”, Govt of India (SAN No. 102/IFD/SAN/864/2018-2019).

Data availability All data generated or analysed during this study are included in this published article.

Compliance with ethical standard

Conflict of interest The authors declare that there is no conflict of interest regarding the publication of this article.

References

- Parkinson GN et al (2002) Crystal structure of parallel quadruplexes from human telomeric DNA. *Nature* 417:876–880
- Siddiqui A et al (2002) Direct evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-MYC transcription. *Proc Natl Acad Sci USA* 99:11593–11598
- Lipps HJ, Rhodes D (2009) G-quadruplex structures: in vivo evidence and function. *Trends Cell Biol* 19:414–422
- Biffi G et al (2013) Quantitative visualization of DNA G-quadruplex structures in human cells. *Nat Chem* 5:182–186
- Mathad RI et al (2011) c-MYC promoter G-quadruplex formed at the 5'-end of NHE III1 element: insights into biological relevance and parallel stranded G-quadruplex stability. *Nucl Acids Res* 39:9023–9033
- Dai J et al (2011) Solution structure of a 2:1 quindoline-c-MYC G-quadruplex: insights into G-quadruplex-interactive small molecule drug design. *J Am Chem Soc* 133:17673–17680
- Simonsson T et al (1998) DNA tetraplex formation in the control region of c-myc. *Nucl Acids Res* 26:1167–1172
- Rankin S et al (2005) Putative DNA quadruplex formation within the human c-kit oncogene. *J Am Chem Soc* 127:10584–10589
- Fernando H et al (2006) A conserved quadruplex motif located in a transcription activation site of the human c-kit oncogene. *Biochemistry* 45:7854–7860
- Dexheimer TS et al (2006) Deconvoluting the structural and drugrecognition complexity of the G-quadruplex-forming region upstream of the bcl-2 P1 promoter. *J Am Chem Soc* 128:5404–5415
- Agrawal P et al (2014) Themajor G-Quadruplex formed in the human BCL-2 proximal promoter adopts a parallel structure with a 13-nt loop in K⁺ solution. *J Am Chem Soc* 136:1750–1753
- Guo K et al (2007) Formation of pseudosymmetrical G-quadruplex and i-motif structures in the proximal promoter region of the RET oncogene. *J Am Chem Soc* 129:10220–10228
- Tong X et al (2011) Solution structure of all parallel G-quadruplex formed by the oncogene RET promoter sequence. *Nucl Acids Res* 39:6753–6763
- Hud NV, Plavec J (2006) The role of cations in determining quadruplex structure and stability. In: Neidle S, Balasubramanian S (eds) *Quadruplex nucleic acids*, vol 23. Royal Society of Chemistry Publishing Cambridge, pp 100–130
- Simonsson T (2001) G-quadruplex DNA structures—variations on a theme. *Biol Chem* 382:621–628
- Tóthová P et al (2014) Formation of highly ordered multimers in G-quadruplexes. *Biochemistry* 53:7013–7027
- Lim KW (2013) Structure of the human telomere in Na⁺ solution: An antiparallel (2+2) G-quadruplex scaffold reveals additional diversity. *Nucl Acids Res* 41:10556–10562
- Víglaský V et al (2011) The first derivative of a function of circular dichroism spectra: Biophysical study of human telomeric G-quadruplex. *Eur Biophys J* 40:29–37
- Wang Y, Patel DJ (1993) Solution structure of the human telomeric repeat d[AG3(TTAG3)3] G-tetraplex. *Structure* 4:263–282
- Ambrus A et al (2006) Human telomeric sequence forms a hybrid-type intramolecular G-quadruplex structure with mixed parallel/antiparallel strands in potassium solution. *Nucl Acids Res* 34:2723–2735
- Luu KN et al (2006) Structure of the human telomere in K⁺ solution: An intramolecular (3 + 1) G-quadruplex scaffold. *J Am Chem Soc* 128(30):9963–9970
- Phan AT et al (2007) Structure of two intramolecular G-quadruplexes formed by natural human telomere sequences in K⁺ solution. *Nucl Acids Res* 35(19):6517–6525
- Marušič M et al (2012) Solution-state structure of an intramolecular G-quadruplex with propeller, diagonal and edgewise loops. *Nucl Acids Res* 40(14):6946–6956
- Neidle S, Parkinson G (2002) Telomere maintenance as a target for anticancer drug discovery. *Nat. Rev. Drug Discov.* 1(5):383–393
- Dai JX et al (2008) Polymorphism of human telomeric quadruplex structures. *Biochimie* 90(8):1172–1183
- Punchihewa C, Yang D (2009) Therapeutic targets and drugs II: G-quadruplex and G-quadruplex inhibitors. In: *Cancer drug discovery and development: telomeres and telomerase in cancer*, vol 23. Hiyama K, pp 251–280
- Qin Y, Hurley LH (2008) Structures folding patterns and functions of intramolecular DNA G-quadruplexes found in eukaryotic promoter regions. *Biochimie* 90(8):1149–1171
- Smargiasso N et al (2008) G-quadruplex DNA assemblies: Loop length, cation identity, and multimer formation. *J Am Chem Soc* 130(31):10208–10216
- Heddi B, Phan AT (2011) Structure of human telomeric DNA in crowded solution. *J Am Chem Soc* 133(25):9824–9833
- Miller MC et al (2010) Hydration is a major determinant of the G-quadruplex stability and conformation of the human telomere 3' sequence of d(AG 3(TTAG3)3). *J Am Chem Soc* 132(48):17105–17107
- Miyoshi D et al (2002) Molecular crowding regulates the structural switch of the DNA G-quadruplex. *Biochemistry* 41(50):15017–15024
- Víglaský V et al (2010) Structural features of intra- and intermolecular G-quadruplexes derived from telomeric repeats. *Biochemistry* 49(10):2110–2120
- Bugaut A, Balasubramanian S (2008) A sequence-independent study of the influence of short loop lengths on the stability and topology of intramolecular DNA G-quadruplexes. *Biochemistry* 47:689–697
- Guedin A et al (2010) How long is too long? Effects of loop size on G-quadruplex stability. *Nucl Acids Res* 38:7858–7868

35. Hatzakis E et al (2010) Thermodynamic stability and folding kinetics of the major G-quadruplex and its loop isomers formed in the nuclease hypersensitive element in the human c-Myc promoter: effect of loops and flanking segments on the stability of parallel-stranded intramolecular G-quadruplexes. *Biochemistry* 49:9152–9160
36. Ong CW et al (2012) Synthesis of bisquinoline-pyrrole oligoamide as G-quadruplex binding ligand. *Tetrahedron* 68:5453–5457
37. Diveshkumar KV et al (2016) Specific stabilization of c-MYC and c-KIT G-Quadruplex DNA structures by indolylmethyleneindanone scaffolds. *Biochemistry* 55:3571–3585
38. Kessler M et al (2003) Anti- and pro-oxidant activity of rutin and quercetin derivatives. *J Pharm Pharmacol* 55:131–142
39. Bhadra K, Kumar GS (2011) Interaction of berberine, palmatine, coralyne, and sanguinarine to quadruplex DNA: a comparative spectroscopic and calorimetric study. *Biochim Biophys Acta* 1810:485–496
40. Halgren TA et al (2004) Glide: a new approach for rapid, accurate docking and scoring. 2. Enrichment factors in database screening. *J Med Chem* 47:1750–1759
41. Sindhikara D, Borrelli K (2018) High throughput evaluation of macrocyclization strategies for conformer stabilization. *Nat Sci Rep* 8:6585–6592
42. Ramachandran B, Sabitha Kesavan S, Rajkumar T (2016) Molecular modeling and docking of small molecule inhibitors against NEK2. *Bioinformation* 12:62–68
43. Webb B, Sali A (2016) Comparative protein structure modeling using modeller. *Curr Protoc Bioinform* 5:1–37
44. Guex N, Peitsch MC (1997) SWISS-MODEL and the Swiss-Pdb-Viewer: an environment for comparative protein modelling. *Electrophoresis* 18:2714–2723
45. Friesner RA et al (2006) Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein-ligand complexes. *J Med Chem* 49:6177–6196
46. Miyoshi D et al (2003) Structural transition from antiparallel to parallel G-quadruplex of d(G4T4G4) induced by Ca²⁺. *Nucl Acids Res* 31:1156–1163
47. Xu Y et al (2006) The new models of the human telomere d[AGGG(TTAGGG)3] in K⁺ solution. *Bioorg Med Chem* 14:5584–5591
48. Miyoshi D, Karimata H, Sugimoto N (2006) Hydration regulates thermodynamics of G-quadruplex formation under molecular crowding conditions. *J Am Chem Soc* 128:7957–7963
49. Sugimoto N, Nakano S, Kotah S, Matsumura A, Nakamura H, Ohmichi T, Yoneyama M, Sasaki M (1995) Thermodynamic parameters to predict stability of RNA/DNA hybrid duplexes. *Biochemistry* 34:11211–11216
50. Lim KW, Amrane S, Bouaziz S, Xu W, Mu Y, Patel DJ, Luu KN, Phan AT (2009) Structure of the human telomere in K⁺ solution: a stable basket-type G-quadruplex with only two G-tetrad layers. *J Am Chem Soc* 131:4301–4309
51. Kondo J, Adachi W, Umeda S, Sunami T, Takenaka A (2004) Crystal structures of a DNA octaplex with i-motif of G-quartets and its splitting into two quadruplexes suggest a folding mechanism of eight tandem repeats. *Nucl Acids Res* 32:2541–2549
52. Tawani A et al (2017) Structural insight for the recognition of G-quadruplex structure at human c-myc promoter sequence by flavonoid Quercetin. *Sci Rep* 7:3600–3612
53. Genheden S, Ryde U (2015) The MM/PBSA and MM/GBSA methods to estimate ligand-binding affinities. *Expert Opin Drug Discov* 10(5):449–461
54. Li J et al (2011) The VSGB 2.0 model: a next-generation energy model for high-resolution protein structure modelling. *Proteins* 79(10):2794–2812
55. Patel DJ et al (2007) Human telomere, oncogenic promoter and 5'-UTR G-quadruplexes: diverse higher order DNA and RNA targets for cancer therapeutics. *Nucl Acids Res* 35:7429–7455
56. Antonacci C, Chaires JB, Sheardy RD (2007) Biophysical characterization of the human telomeric (TTAGGG)₄ repeat in a potassium solution. *Biochemistry* 46:4654–4660
57. Luu KN, Phan AT, Kuryavyi VV, Lacroix L, Patel DJ (2006) Structure of the human telomere in K⁺ solution: an intramolecular (3 + 1) G-quadruplex scaffold. *J Am Chem Soc* 128:9963–9970
58. Bhattacharjee S, Chakraborty S, Sengupta PK, Bhowmik S (2016) Exploring the interactions of the dietary plant flavonoids Fisetin and Naringenin with G-quadruplex and duplex DNA, Showing contrasting binding behavior: spectroscopic and molecular modeling approaches. *J Phys Chem B* 120:8942–8952
59. Vinnarasi S, Radhika R, Vijayakumar S, Shankar R (2019) Structural insights into the anti-cancer activity of quercetin on G-tetrad, mixed G-tetrad, and Gquadruplex DNA using quantum chemical and molecular dynamics simulations. *J Biomol Struct Dyn* 38:1–23
60. Tawani A, Mishra SK, Kumar A (2017) Structural insight for the recognition of G-quadruplex structure at human c-myc promoter sequence by flavonoid Quercetin. *Sci Rep* 7:1–13
61. Tawani A, Kumar A (2015) Structural insight into the interaction of flavonoids with human telomeric sequence. *Sci Rep* 5:1–13
62. Sun H, Tang Y, Xiang J, Xu G, Zhang Y, Zhang H, Xua L (2006) Spectroscopic studies of the interaction between quercetin and G-quadruplex DNA. *Bioorg Med Chem Lett* 16:3586–3589
63. Mondal S, Jana J, Sengupta P, Jana S, Chatterjee S (2016) Myricetin arrests Human Telomeric G-quadruplex structure: A new mechanistic approach as an anticancer agent. *Mol Biosyst* 12:2–45
64. Sengupta B, Pahari B, Blackmon L, Sengupta PK (2013) Prospect of bioflavonoid fisetin as a quadruplex DNA ligand: a biophysical approach. *PLoS ONE* 8:1–45

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.